EXHIBIT D

DiCarlo, et al., *A Qualitative Evaluation of the Effect Cleaning Products Have on the Bluestar Test for Latent Blood*

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A Qualitative Evaluation of the Effect Cleaning Products Have on the Bluestar Test for Latent Blood

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Abstract:

In violent crimes, blood is one of the most common physical evidence that may be found. However, with the interest of forensic investigation being on the forefront for many years, criminals have become more knowledgeable about the necessity of cleaning their scene. Due to the attempted clean up, the effects of household chemical cleaners on presumptive tests might cause false positive or negative results. In order to assess this, cleaners (Clorox, Green Works, Lysol, and Windex) were used on floor surfaces (carpet, ceramic tile, and press-on vinyl tile), and their effects were qualitatively examined with relation to the Bluestar test for latent blood detection. This resulted in no effect by Windex and Green Works, a false-positive by Clorox, and a false-negative by Lysol.

Keywords: Blood detection, Blood detection change, Bluestar, Clean up, Clean up effects, Crime Scene, Forensic Science, Household cleaners.

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Introduction

Blood is one of the most common types of physical evidence in violent crimes, and its analysis can provide very useful information.[1] Today, there are many presumptive tests for the detection of this very important bodily fluid, including the forensic luminol test.[1] Luminol has been used in the field of forensic sciences for over 60 years as a presumptive test for blood stains, starting from its creation by Walter Sprech in 1937.[1] This chemiluminescent product has enabled investigators to visualize, evaluate, and collect latent, or invisible, bloodstains.[2]

Chemiluminescence is the production of light from a chemical reaction.[3] The reactants form a high-energy intermediate, which breaks down releasing some energy as photons, a quantum of light energy.[3] When these photons have a wavelength that is found in the range of visible light, the change from high-energy intermediate to ground level is perceived as light of a particular colour.[3]

The chemiluminescence of luminol is based directly on the availability of haemoglobin in bloodstains and creates a blue-green colour with no need for a light source.[1] Since luminol is a presumptive test it is clear that it can release chemiluminescence without the presence of haemoglobin producing a false-positive result.[4]

Bluestar is a luminol based presumptive reagent for the detection of latent bloodstains. However, the properties of this reagent make it more convenient to use on crime scenes.[2] Unlike luminol it does not require complete darkness and it can be sprayed several times without an effect on its chemiluminescence.[2] Dr. Loic Blum discovered this solution in 2000.[2] Bluestar takes on the mechanism depicted in Figure 1 to product chemiluminescence with blood. Chemiluminescence requires a catalyst, which in the case of luminol and luminol based products, is the iron in the haemoglobin.[2]

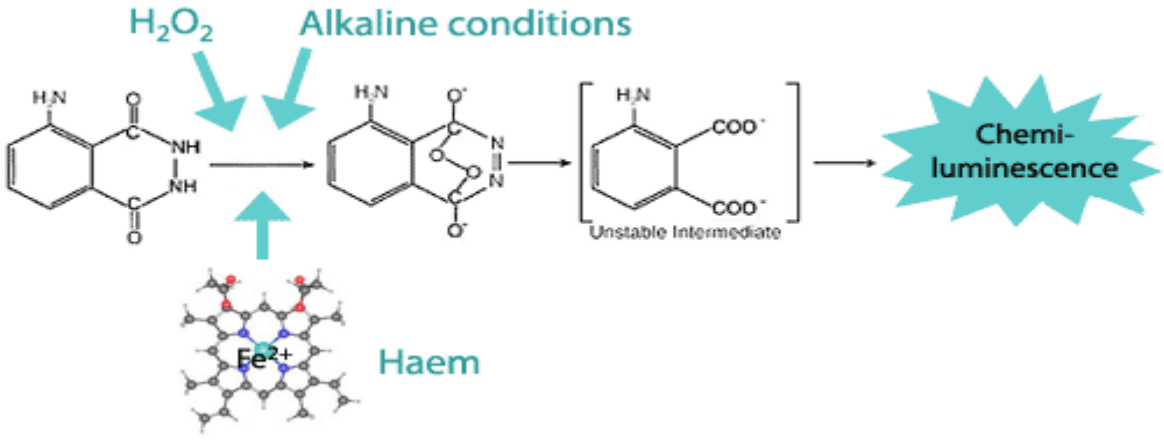

Figure 1. Reaction mechanism of Bluestar with haemoglobin from blood.

J.I. Creamer, et al, completed an experimentation of the interferences on the luminol test for blood in 2003.[4] To do so a wide variety of substances were chosen and the chemiluminescence of each was tested with luminol to determine the shift in peek chemiluminescence.[4] This was done very thoroughly for luminol, however only resultants giving a change were released.[4]

In the experiment by A. Nilsson, it is acknowledged that cleaning can affect the chemiluminescence of luminol.[1] However, it is not shown past the use of bleach-based cleaners.

Lisa Dilbeck completed an experimentation in which she compared luminol to Bluestar. With this experimentation it was determined that Bluestar has distinct advantages in comparison.[2] These advantages were determined in the following categories: ease of mixing, lack of complete darkness, and intensity after initial spray.[2] Moreover, Dilbeck noted that Bluestar is as sensitive in detecting dilute concentrations of blood as luminol.[2] However, where this study fell short was in determining the limitations of this presumptive reagent and there was no reference to any effects brought on by Bleach (the only household cleaning product used).

In the following study the effects of four common household cleaners will be tested with the Bluestar test for latent blood. By focusing strictly on cleaning products, the research will aid in determining if a crime scene has been cleaned of blood. Since this has not yet been an exclusive study in the past, it will be completed qualitatively. In that regard, it will expand the knowledge we have on the effects of clean up on the visualization of latent blood and will go to directly show how each product changes the chemiluminescence to the naked eye, as well as to a cameras exposure. This will therefore determine whether specific cleaners will cause a false-positive, false-negative, or have no effect on the Bluestar test for latent blood detection.

Materials and Methods

In order to complete the laboratory experiment one used the household cleaners Lysol Power & Free Multi-Purpose Cleaner With Hydrogen Peroxide, Green Works All Purpose Cleaner, Clorox Clean-Up Disinfectant Bleach Cleaner, and Windex Original Glass & More Cleaner With Ammonia-D, four ceramic tiles, four vinyl stick on tiles, one bathroom rug cut into four sections, six multi-purpose cellulose sponges cut in half, 2 Bluestar kits, water, spray bottle, blood, and tape.

In starting the experiment one created the test surfaces. To do so, each tile and carpet piece was divided into four sections using strips of tape to visually show the division. Blood was then taken into a sponge and impacted onto the three of the four quadrants and left for 24 hours minimum to dry (as per T.I. Quickenden and Paul D. Cooper).[5] Following this drying period, two quadrants of blood and the blank section were wiped with one of the household cleaners such that the test surfaces had the following pattern depicted in Figure 2.

Blank- wiped once with cleaner (Q1)	Blood- not cleaned (Q2)
Blood- wiped once with cleaner (Q3)	Blood- fully cleaned with cleaner (Q4)

Figure 2. Test surface set up

Q1 and Q2 act as a control where Q3 and Q4 are the experimental portions. Q3 is wiped only once such that blood is still visible to the naked eye where Q4 is representative of the area cleaned in a way that the naked eye cannot see any residual blood. Following this cleaning, the test surfaces were left in order to dry for a minimum of 2 hours (in this time, carpet samples were repositioned half way though to move from wet spot created by cleaner seeping onto table). In the drying time the luminol solution was created. To do so one followed the instructions on the kit.

They read,

> "1. Open spray bottle; add 125mL (4 fl. Oz) of distilled water. Then add a pair of BLUESTAR FORENSIC "TRAINING" tablets. If you need more working solution use 125mL (4 fl. Oz) per pair of tablets.
> 2. Mount the plunger onto the spray bottle head and screw on the head of the bottle firmly.
> 3. Allow about 1 to 2 minutes for complete dissolution and mixing of chemicals, stirring gently with a circular motion of your hand. Do NOT shake the container upside down."

Following the completion of the drying, test surfaces were sprayed with the Bluestar solution. Once sprayed, the results were photographed using a shutter speed of 30 seconds, and F13 stop with an ISO of 800. These methods were followed for all 12 of the test surfaces.

Results

In completing the initial tests with the Clorox cleaning product it was shown in Q1, the cleaner control, that there was streaking. This was mainly observed in person, however is not as notable in the photographs captured. These tests are depicted in Figure 3, Figure 4, and Figure 5.

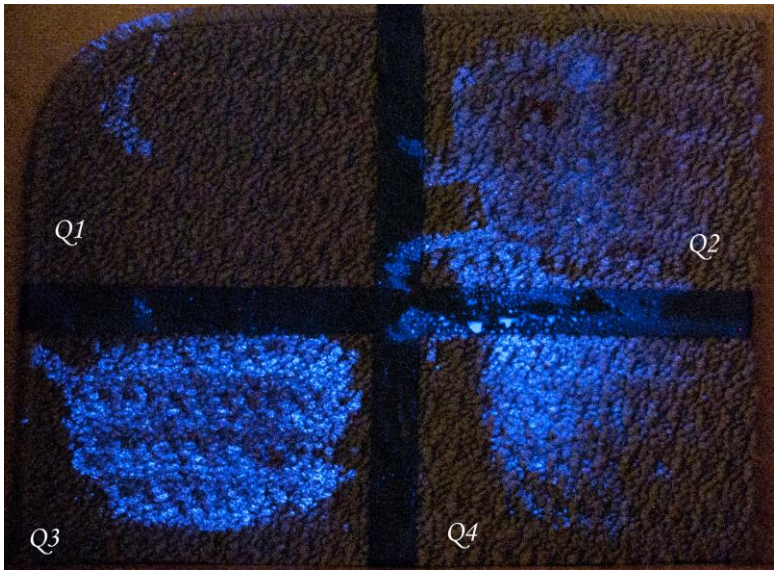

Figure 3. Carpet cleaned with Clorox

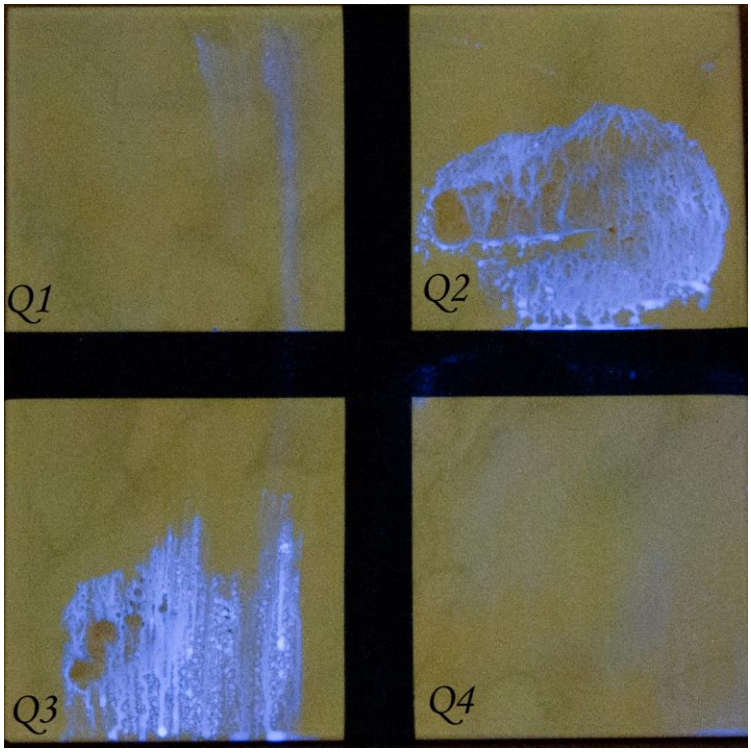

Figure 4**.** Ceramic tile cleaned with Clorox

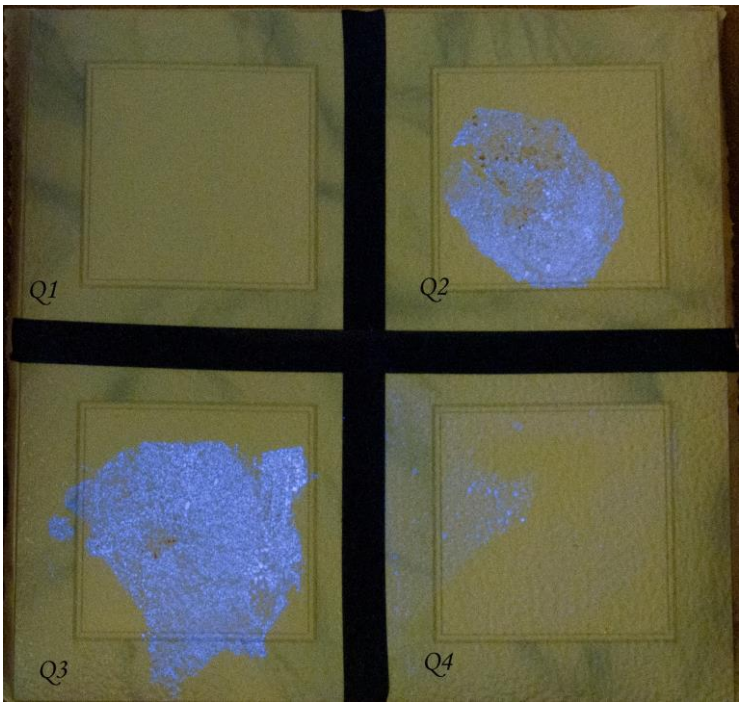

Figure 5. Press on tile cleaned with Clorox

The next tests were completed using Lysol. In Q3, the blood wiped once with cleaner, there is hints of the brightness being depleted when compared to Q2, the blood control. However when viewed, the naked eye detected no light in the Q3 section. This is depicted in Figure 6, Figure 7, and Figure 8.

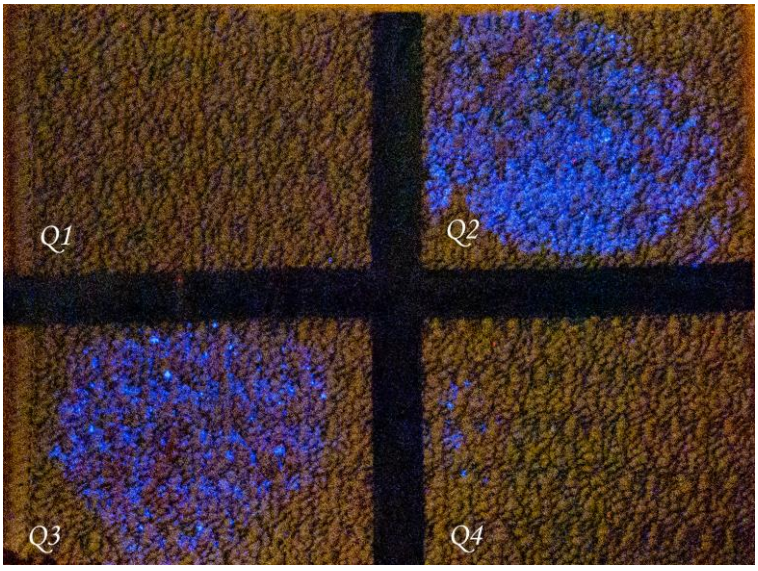

Figure 6. Carpet cleaned with Lysol

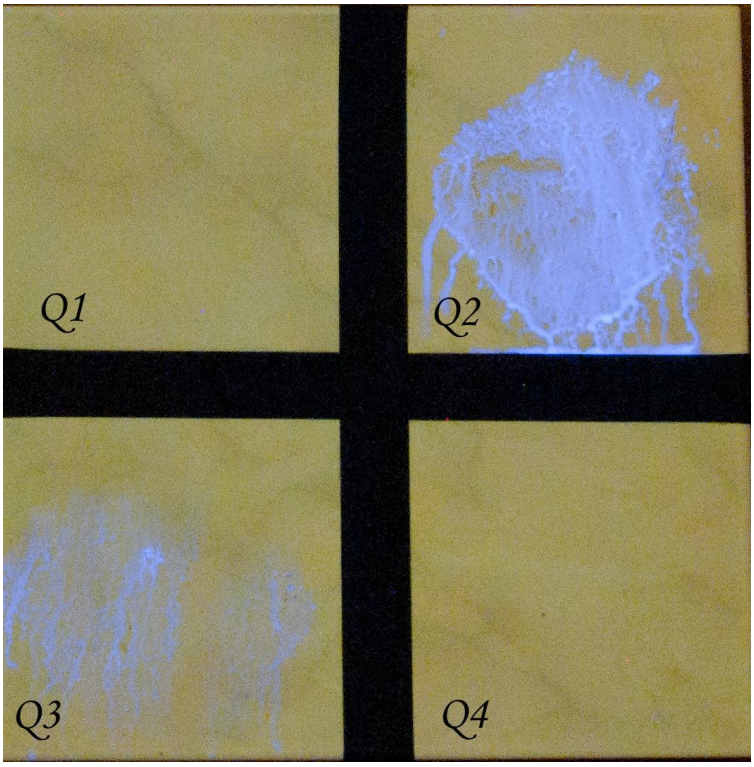

Figure 7. Ceramic tile cleaned with Lysol

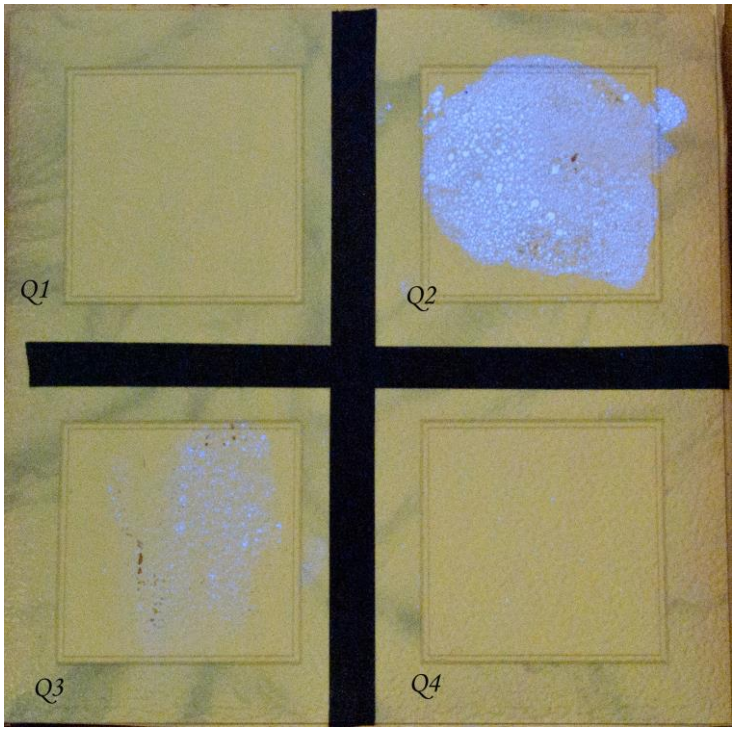

Figure 8. Press on tile cleaned with Lysol

Finally in the trials with Green Works and Windex it can be noted that there is no difference in the appeared brightness between Q2, Q3, and Q4, the blood wiped visibly away with cleaner in the figures Figure 9, Figure 10, Figure 11, Figure 12, Figure 13, and Figure 14. However, in Q1 of Figure 12, Figure 13, and Figure 14 some streaking is noted in the photographs that were not visible in person.

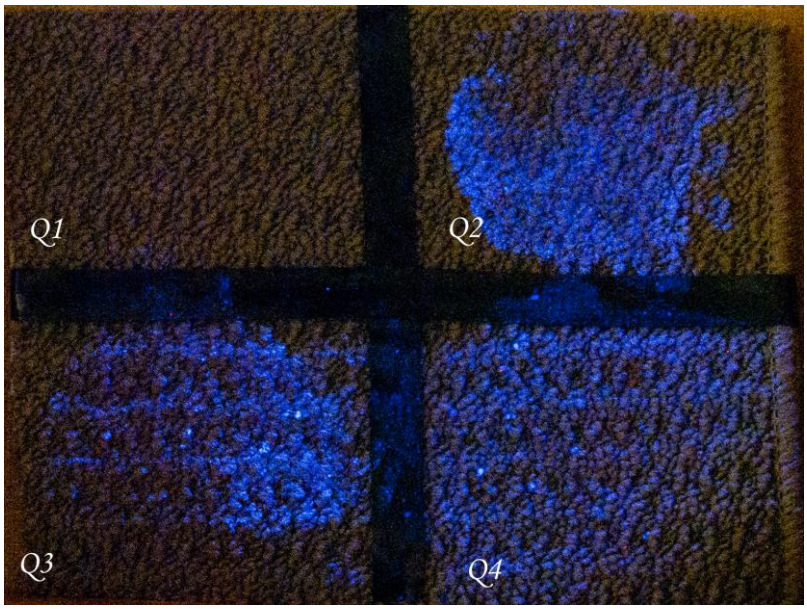

Figure 9. Carpet cleaned with Green Works

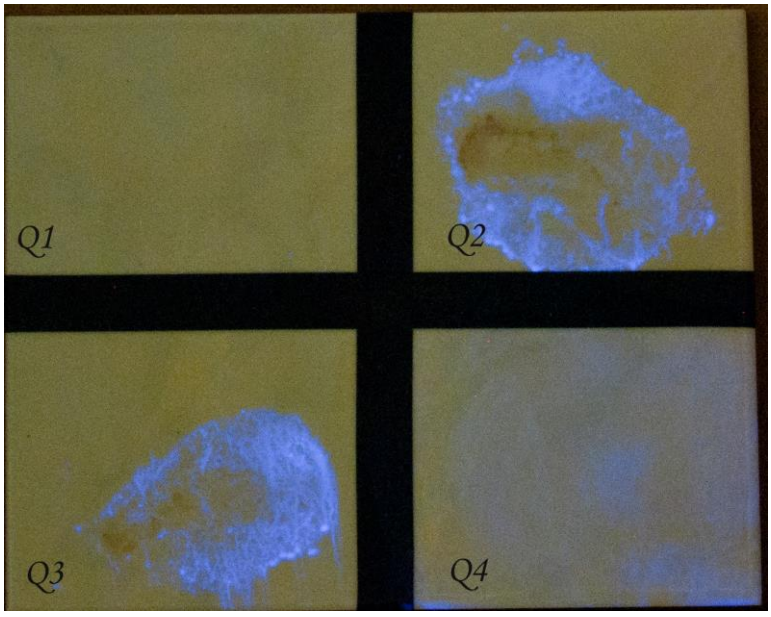

Figure 10. Ceramic tile cleaned with Green Works

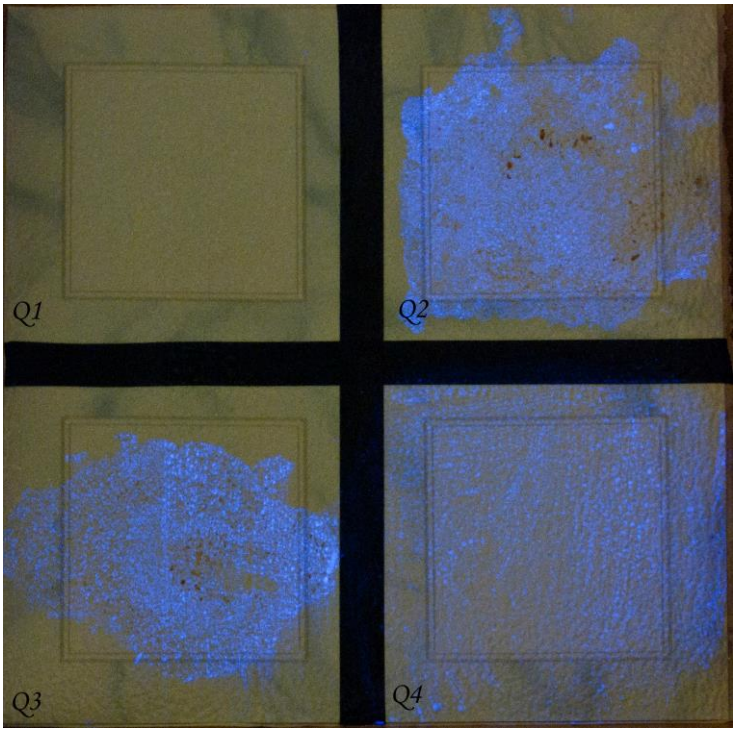

Figure 11. Press on tile cleaned with Green Works

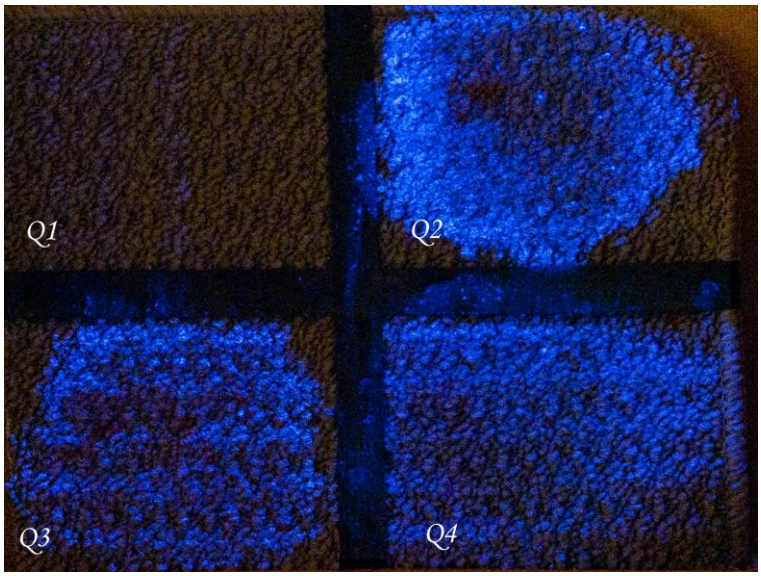

Figure 12. Carpet cleaned with Windex

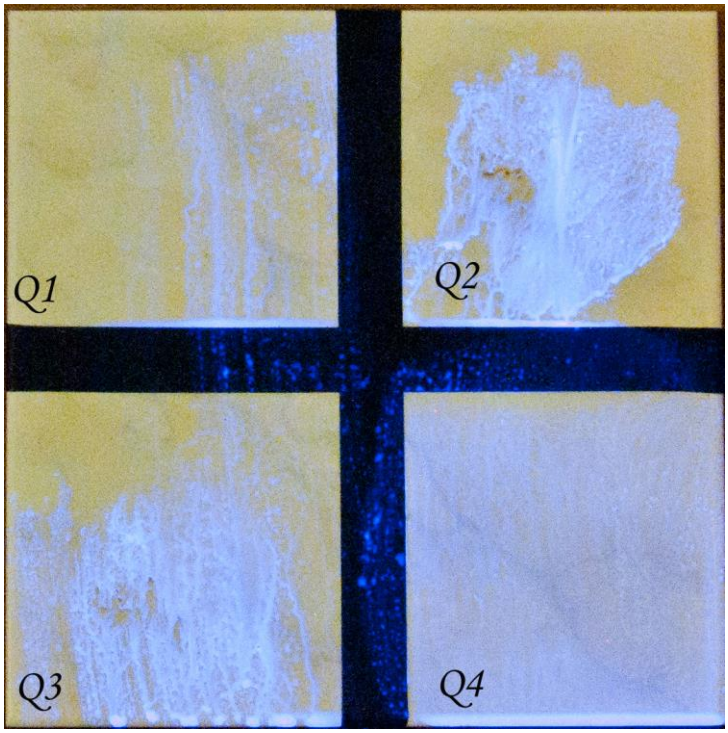

Figure 13, Ceramic tile cleaned with Windex

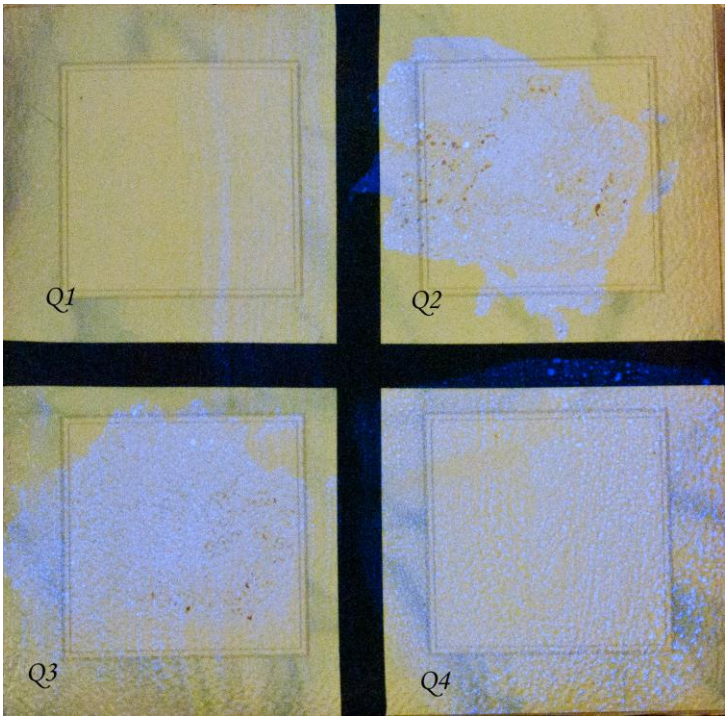

Figure 14. Press on tile cleaned with Windex

Discussion

After viewing the resultant images there is a clear difference between the cleaners. In the experiments completed using Green Works and Windex it is noted that there is no difference between Q2, Q3, and Q4. This gives rise to these cleaners not having an effect on the Bluestar test for latent blood detection. However, in Q1 of the Windex trials streaking can be viewed in the images. At this stage of the experimentation there can be no comment made on why this has occurred.

In the experiments cleaned with the Lysol there was a clear difference between Q2 and Q3 in Figure 6, Figure 7, and Figure 8. In these images it is noted that Q2 is significantly brighter than Q3. This was extremely visible to the naked eye where no chemiluminescence was visible in the Q3 section. Since the light entering a cameras sensor is additive the slight chemiluminescence is shown more in the resultant images. This decrease in chemiluminescence is a slight false-negative result showing that the use of Lysol will affect the Bluestar test. An experiment by Enika Nagababu and Joseph Moses Rifkind in 2000 shows a reaction between hydrogen peroxide and the haem

$$\mathrm{Hb(II) + H_2O_2 \rightarrow HbFe(IV){=}O + H_2O_2 \rightarrow}$$
$$\mathrm{HbFe(III) + O_2^{\bullet -} \rightarrow \text{heme degradation}}$$

Figure 15. Haem degradation by hydrogen peroxide [6]

group in blood.[6] This can be modeled by the reaction mechanism in Figure 15. Because of the hydrogen in the Lysol cleaner degrades the haem group in the blood it quenches, or partly quenches, the ferryl haem needed to catalyze the reaction with Bluestar. This means there will be little to no chemiluminescence produced after cleaning.

This is the reverse of what happens with the Clorox cleaner in Figure 3, Figure 4, and Figure 5. In these images it is noted there is streaking in Q1 as well as slight brightening of Q3 when compared to Q2. In the observation of the experiment taking place the streaking was very bright for a short amount of time. This is indicative of false-positive reaction. These results are consistent with testing completed by Dilbeck and Nilsson.

Conclusion

It can be concluded that the results obtained were successful in determining the false-positives or false-negatives due to household cleaners on the Bluestar test for latent blood. This was observed through using three test surfaces and cleaning blood stains off of them with household cleaners. It was shown that Lysol has a false-negative result, Clorox has a false-positive result, and Green Works and Windex show no change on the Bluestar test.

Acknowledgements

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